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Dose-dependent increases in markers of hepatorenal damage in rats treated with different doses of virgin engine oil

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ABSTRACT:
Exposure to chemicals is capable of initiating abnormal clinical presentations. In most cases the degree of exposure determines whether toxicity-enhancing processes (usually at play after toxic agent administration) overwhelm toxicity-preventing ones. The study is embarked upon to compare the degree of toxicity that is associated with two doses (0.5 and 1.0 mL/kg body weight) of engine oil exposure, after 30 days of daily administration. Thirty adult female rats (200-240 g) were divided equally into five experimental groups. The first and second groups were treated with 0.5 and 1.0 mL/kg through the dermal route respectively while third and fourth groups were treated with 0.5 and 1.0 mL/kg through the oral route respectively. The fifth group served as the control. Serum obtained from blood collected by retro-orbital bleeding was stored at - 20ºC until required for analysis. Serum levels or activities of hepatorenal markers (alanine/aspartate aminotransferase, alkaline-phosphatase, γ- glutamyl transferase, total-protein, albumin, urea, creatinine) were investigated using standard techniques. The mean values of all estimated parameters were analyzed using analysis of variance, $p \leq 0.05$ was considered significant. Results revealed that both doses caused significant differences in most of the markers (urea, creatinine, albumin, globulin, hepatic enzymes) but the degree of alteration was more pronounced in the higher dose of 1.0 mL/kg than lower dose of 0.5 mL/kg. Data obtained through the study suggest that even low dose exposure can result in hepatorenal toxicity. Therefore exposure to engine oil from environmental contamination, occupational contact or deliberately for cosmetics/ therapeutic reasons should be avoided.

Keywords: virgin engine oil; dermal & oral routes, low & high dose-exposure; liver; kidney.

INTRODUCTION

Studies abound that have been conducted to investigate the effect of engine oil on man and many experimental animals, but in most cases these studies have been directed at used engine oil. This is because such exposure is global and occurs in different classes and categories of workers. Examples of human subjects that are included in these categories are auto-mechanics in the course of automobile repair; resource-scarce cattle farmers who are exposed to engine oil in the course of tick management; and in many parts of the developing world- general populace who come in contact with engine oil from indiscriminate discharge into the environment [1-3]. Yet exposure to engine oil is not restricted to the used type, contact with virgin oil is also common. This is especially possible in the Nigerian environment as there are reports which indicate that there is proliferation of sale outlets for many petroleum products even the highly flammable ones. In addition, there are claims that suggest therapeutic usefulness of not only kerosene and petrol [1] but of virgin engine oil. As a result of this, possible harmful effect of virgin engine oil is being determined through this study.

Xenobiotics are substances foreign to a system and therefore capable of initiating a number of processes that may be beneficial or harmful. While orthodox therapeutic agents are known for their beneficial effects, many other agents that have been adapted for therapeutic purposes for which they are not meant may provoke catastrophic events at the molecular level. Virgin engine oil is principally made up of aliphatic, aromatic, and many branched saturated and unsaturated hydrocarbons [4] Yet fresh engine oil contain additives, these are compounds that are employed in the oil formulation so as to enhance physicochemical properties of the oils [5]. Different combinations of additives are necessary to meet the required performance level, especially in their role as detergents, dispersants, antiwear, antioxidants, viscosity modifiers, foam inhibitors, and pour point depressants [6]. Many times, these additives compounds contain heavy metals such as Ni, Al, Pb and Sn [7,8] and because of the harmful effects of heavy metal on many organs in the body, the objective of this study is to determine the impact of constant exposure to virgin engine oil on hepatic and renal cells in Wistar rats.

MATERIALS AND METHODS

All the chemicals used for the study were of analytical grade and were supplied by Sigma-Aldrich®. On the other hand, engine oil was purchased from a filling station located in Osogbo, Osun State, Nigeria in December, 2011.

Experimental Animals and design

This study was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). Adult female albino rats weighing between 200-240 g obtained from the Animal House attached to the Department of Veterinary Physiology, University of

Ibadan, Nigeria were employed for the study. Before the experiment commenced the animals were left to acclimatize for two weeks. Animals were housed in cages at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle. All the animals were given unrestricted access to water and feed.

The study consisted of five experimental groups; the first and second groups consisted of rats exposed to 0.5 and 1.0 mL engine oil/kg body weight through the dermal route respectively. The third and fourth groups were made up of rats treated with 0.5 and 1.0 mL of engine oil/kg body weight through the oral route respectively. The fifth group served as the control. Each group consisted of six rats. Exposure to engine oil through the oral route occurred as contaminant of feed. In which engine oil was mixed thoroughly with the feed daily whereas exposure to those in dermal group was directly to the skin. The experiment was for duration of thirty days. Blood was collected from each rat by retro-orbital bleeding, dispensed into anti-coagulant free bottle, and centrifuged at 3000 g for ten minutes. The serum obtained was stored at -20ºC until required for analysis.

Clinical Chemistry

Serum concentrations of total proteins, creatinine, and urea were assessed using Biuret method, Jaffé reaction and diacetyl monoxime oxidase method respectively. Activities of liver aspartate and alanine aminotransferases were determined using Bergmeyer *et al*. [9] method, whereas that of serum alkaline phosphatase (ALP) was estimated using the Mc Comb and Bowers method [10]. The serum levels of bilirubin and albumin were carried out using modified Jendrassik-Groff [11] & standard bromocresol methods respectively. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for these estimations.

Statistical analysis

The mean values of the serum levels of the markers of hepatic and renal function were compared using the analysis of variance (ANOVA) Statistical Package for Social Sciences was employed for this purpose. Value of $p \leq 0.05$ was considered significant.

RESULTS

Results of the study are presented in **Tables 1-3** below. Administration of virgin engine oil at 1.0 mL/kg BW (body weight) to Wistar rats resulted in significant decrease ($p < 0.05$) in the serum concentrations of albumin, significant increases in globulin level, but both bilirubin and total protein were not significantly changed (**Table 1**). On the other hand administration at 0.5 mL/kg BW caused no significant change in the serum levels of albumin, globulin, total protein and bilirubin ($p>0.05$) as presented in **Table 1**.

As shown in **Table 2,** the activities of all the hepatic enzymes estimated (namely AST, ALT, ALP, γ-GT) were significantly different at both exposure levels i.e. 0.5 mL/kg BW and 1.0 mL/kg BW ($p>0.05$). Moreover, as shown in **Table 3** results of markers of renal damage were significantly different ($p<0.05$) at both levels of exposure.

DISCUSSION

The degree of toxicity of an agent depends primarily on the concentration and persistence of the ultimate toxicant at its site of action as shown by the results of the present study. In which high dose exposure-level resulted in a significantly higher concentrations of hepatic and renal markers than lower dose exposure-level. It seems that the ultimate toxicant (the chemical species) combined with the endogenous target molecule (e.g., receptor, enzyme, DNA, microfilamental protein, lipid) or altered the biological (micro) environment, thereby provoking structural and/or functional changes which eventually lead to hepatorenal toxicity. While it is generally believed that the ultimate toxicant is a metabolite of the parent (toxic) compound or a reactive oxygen or nitrogen species produced during the biotransformation of the toxicant, there are occasions in which the original chemical itself exerts the toxic effects. Aliphatic and aromatic compounds contained in engine oil may be such examples that are responsible for the toxic effects observed in both hepatic and renal cells. Ita and Udofia [1] have suggested that aliphatic and aromatic hydrocarbons that are the major components of crude petroleum and petroleum products result in generation of free radical species in various tissues. Although it cannot be ruled out that some other components of engine are capable of binding directly to cellular macromolecules to induce tissue toxicity.

Usually, the level of the ultimate toxicant at the target site (molecules) determines the degree of toxicity, and this is also determined by relative effectiveness of the different pathways or processes that increase or decrease its concentration at the target site. These pathways or processes can be classified into two; which are toxicity-enhancing and toxicity-depressing processes. Toxicity-enhancing processes include absorption, distribution to the site of action, reabsorption, and metabolic activation of toxicant. Yet it is important to note that toxicity-depressing processes such as presystemic elimination, distribution away from the site of action, excretion, and detoxification that oppose toxicity-enhancing ones work against the accumulation of the ultimate toxicant at the target

molecule and therefore also influence the degree of toxic effects of a xenobiotic. From results obtained through this study, it seems as if toxicity depressing processes were not effective to counteract the toxic impact of engine oil in Wistar rats at 1.0 mL/kg level of exposure as significant increases in the indices of hepatorenal axis were observed.

Apart from the kidney being a rich source of the enzymes responsible for xenobiotics metabolism, its unusual susceptibility to the toxic effects of noxious chemicals may not be unassociated with the unique physiologic and anatomic features of this organ. While it is known that the kidney is responsible for only 0.5% of total body mass, both kidneys receive about 20–25% of the resting cardiac output. This means that any chemical agent or drug in the systemic circulation will be delivered to the kidney in relatively higher proportion compared to many other organs of the body. This may be the reason why a wide range of drugs, environmental chemicals, and metals can cause nephrotoxicity [12], and also a possible explanation for the nephrotoxic effects of engine oil.

Aside this, the different processes involved in concentrated urine formation also invariably serve to concentrate potential toxicants in the tubular fluid (American Society of Nephrology, 2005). Re-absorption of water and electrolytes from the glomerular filtrate equally causes chemicals in the tubular fluid to be concentrated, a situation capable of driving passive diffusion of toxicants into tubular cells. The implication of this is that a nontoxic concentration of a chemical in the plasma (or many other tissue), may reach toxic levels in renal cells. This probably explains why exposure of a toxic agent to an animal causes different presentation to various organs.

The significant increases in the levels or activities of markers of hepatorenal function portray liver and kidney damage. It is an established fact that in some cases progressive concentration of toxicants along the nephron causes intraluminal precipitation of relatively insoluble compounds, thereby provoking acute renal failure that is secondary to tubular obstruction [13]. While the results of this study did not identify if the damage done to the kidney is primary to both the glomerulus and tubules, studies have shown that glomerulus may be the initial site of chemical exposure within the nephrons. Many chemicals have been reported to produce structural injury to this segment. Aside outright damage, xenobiotics alter glomerular permeability to proteins by altering the size- and charge-selective functions. For example, cyclosporine- a nephrotoxic agent- causes renal vasoconstriction and vascular damage as well as injury to the glomerular endothelial cell [14,15].

Chemical injury induced by chemical agents has also been reported to be mediated by extrarenal factors. Processes such as formation of immune complexes may result in entrapment of circulating immune complexes within the glomeruli thereby causing binding of complement, attraction of neutrophils, and phagocytosis. That may also not be ruled out in this case, although jet oil another petroleum product has been identified to provoke a suppression of immune response [16]. Schnellmann [17] has indicated that neutrophils and macrophages within glomeruli is a common manifestation of membranous glomerulonephritis, since this is also accompanied by local release of cytokines and reactive oxygen species (ROS), it is likely to contribute to glomerular injury. Heavy metals such as $HgCl_2$, gold and cadmium, (many heavy metals have also been identified in petroleum products) can induce this type of glomerular injury.
Chemical-induced renal injury has also been linked to the proximal tubule which is usually the most common site of toxicant-induced nephrotoxicity [18]. This has been associated partly to the selective accumulation of xenobiotics into this segment of the nephrons. In addition, the proximal tubule also possesses a leaky epithelium that favors the flux of compounds into proximal tubular cells; this is unlike the distal tubule that is characterized by a relatively tight epithelium with high electrical resistance.

The results of hepatic markers showed significant increases in all groups of treated rats when compared with the control, although the level of orally treated rats were higher than in rats in dermal routes of exposure. Markers of hepatic damage namely AST and ALT were significantly increased signifying hepatic membrane damage. Interestingly, serum bilirubin was not significantly increased which suggest that the excretory function was not profoundly affected. It had earlier been suggested that even with destruction of 50% of hepatocytes, the liver may still be able to carry out some of its metabolic functions.

The implication of this is that humans that are exposed to this agent in significant amount may present with abnormality of liver and renal function, this is because of homology in the genetic constitution of many mammals. These results suggest that many of the central and critical biochemical roles in the metabolism, digestion, detoxification and elimination of substances from the body played by this organ will be adversely affected [19]. The fact that ALP and γ-GT were significantly increased raises the possibility that the

impact of engine oil goes well beyond destruction of membrane of the hepatocytes. The non-significant difference in serum total protein level may be ascribed to the fact that both albumin and globulin were significantly different, with globulin being significantly increased and albumin being significantly decreased. The significant decline in the level of serum albumin in rats in the treatment groups compared with control suggest an abnormality of hepatic function as well as the involvement of the inflammatory process. According to Dafour [19], interleukin-6, a molecule closely linked with inflammatory process, increases the synthesis of acute phase protein but reduces albumin synthesis. The liver and kidney are susceptible tissue because of the nature of their endothelial cells; endothelial cells in the hepatic sinusoids and in the renal peritubular capillaries have larger fenestrae that allow the transfer of even protein-bound xenobiotics, a process that favors the accumulation of chemicals in the liver and kidneys.

Table 1: Serum levels of bilirubin, total protein, albumin and globulin of engine oil-administered and control rats.

	High dose exposure (1.0 ml/kg)				Low dose exposure (0.5 ml/kg)			
	Bilirubin (µmol\L)	Total protein (g\L)	Albumin (g\L)	Globulin (g\L)	Bilirubin (µmol\L)	Total protein (g\L)	Albumin (g\L)	Globulin (g\L)
Control	9.17±0.38	75.07±3.05	39.63±1.04	35.94±1.05	9.17±0.68	75.07±5.05	39.63±2.04	35.94±1.05
Oral route	10.08±0.61	78.33±4.02	33.21±1.05	45.12±0.97	9.35±0.35	75.99±6.64	37.24±6.05	38.75±4.05
Dermal route	9.92±0.40	77.32±2.64	36.01±2.22	41.31±1.09	9.01±0.60	74.32±4.23	37.52±2.71	36.80±1.83
F value	0.155	0.657	17.273	42.17	0.098	0.461	4.019	0.746
P value	0.801	0.542	0.010*	0.003*	0.838	0.536	0.057	0.297

Results are expressed as mean ± standard error of mean. *P < 0.05 is significant when control, dermal and oral groups compared using ANOVA, n=6.

Table 2: Activities of hepatic enzymes of engine oil-administered and control rats

	High-dose exposure (1.0 ml/kg)				Low-dose exposure (0.5 ml/kg)			
	AST (IU\L)	ALT (IU\L)	γ-GT (IU\L)	ALP (IU\L)	AST (IU\L)	ALT (IU\L)	γ-GT (IU\L)	ALP (IU\L)
Control	24.56±1.96	30.06±1.51	34.60±2.03	40.33±3.27	24.56±3.96	30.06±2.51	34.60±2.03	40.33±3.27
Oral route	94.54±4.25	83.79±3.87	46.85±1.22	71.00±1.99	88.72±1.79	50.13±2.56	54.50±3.04	60.38±1.39
Dermal route	59.25±2.81	46.70±1.36	40.11±1.27	48.56±3.24	58.86±0.90	37.06±1.63	38.09±0.36	49.45±1.08
F value	25.122	60.005	118.680	23.503	63.980	90.518	188.641	17.098
P value	0.005*	0.021*	0.007*	0.016*	0.014*	0.032*	0.022*	0.002*

Results are expressed as mean ± standard error of mean. *P < 0.05 is significant when control, dermal and oral groups compared using ANOVA, n=6. Abbreviations: ALT-alanine aminotransferase; AST- aspartate aminotransferase; ALP- alkaline phosphatase; γ-GT γ-glutamyl transferase.

Table 3: Serum levels of urea and creatinine of engine oil-administered and control rats.

	High dose exposure (1.0 ml/kg)		Low dose exposure (0.5 ml/kg)	
	Urea (mmol\L)	Creatinine (μmol\L)	Urea (mmol\L)	Creatinine (μmol\L)
Control	4.64.22±0.75	57. 17±10.03	4.64.22±0.75	57. 17±10.03
Oral route	11.02±0.83	90.11±9.86	7.44±36	76.06±6.61
Dermal route	7.99±0.74	71.34±5.47	5.16±09	64.51±3.17
F value	51.780	316.592	5.318	41.337
P value	0.009*	0.001*	0.014*	0.006*

Results are expressed as mean ± standard error of mean. *P < 0.05 is significant when control, dermal and oral groups compared using ANOVA, n=6.

CONCLUSION:

Virgin engine oil caused significant alterations to markers of renal and hepatic function in Wistar rats treated with both 0.5 and 1.0 mL/kg levels of exposure. Every effort should therefore be exercised to ensure that exposure of humans to engine oil is brought to a possible minimal level. In cases when contact with engine oil cannot be avoided, it is advisable that protective clothing that will prevent skin contact should be worn.

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REFERENCES

[1] Ita S.O., Udofia U.A. (2011) Comparative study of some haematological parameters in rats following ingestion of crude oil (Nigerian Bonny Light), petrol, kerosene and diesel. Asian Jour Biol Sci, 4:498-505.

[2] Moyo B., Masika P.J. (2009) Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. Trop Anim Health Prod, 41(4):517-23.

[3] Patrick-Iwuanyanwu K.., Ogwe G., Onwuka F. (2009). The hepatotoxic effects of the water-soluble fraction of spent lubricating oil in wistar albino rats. The Internet Jour Toxicol, 7(2): .

[4] Hatanaka R.R., Sequinel R, Gualtieri C.E., Tercini A.C., Flumignan D.L., de Oliveira J.E. (2013) Development and validation of an environmentally friendly attenuated total reflectance in the mid-infrared region method for the determination of ethanol content in used engine lubrication oil. Talanta, 109:191-6.

[5] Wang H., Lin Z.N., Wu B.L. (2013) Concentration levels and sources analysis of additives element in engine oil. Guang Pu Xue Yu Guang Pu Fen Xi, 33(9):2579-82.

[6] Rahimi B., Semnani A., Nezamzadeh-Ejhieh A., Langeroodi H.S., Davood M.H. (2012) Monitoring of the Physical and Chemical Properties of a Gasoline Engine Oil during Its Usage. J Anal Methods Chem, doi: 10.1155/2012/819524.

[7] Kim Y., Kim N.Y., Park S.Y., Lee D.K., Lee J.H. (2013) Classification and individualization of used engine oils using elemental composition and discriminant analysis. Forensic Sci Int, 230(1-3):58-67.

[8] Chen L.D. (2013) Application of atomic absorption spectrometry in the engine knock detection. Guang Pu Xue Yu Guang Pu Fen Xi, 33(2):540-3.

[9] Bergmeyer H.U., Scheibe P., Wahlefeld A.W. (1979) Methods for aspartate and alanine amino transferase. Clin Chem, 125:1487.

[10] McComb R.B., Bowers Jr G.N. (1972) A study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. Clin Chem, 18:97.

[11] Koch T.R., Doumas B.T. (1982) Bilirubin: Total and conjugated, modified Jendrassik- Grof method. Am Ass Clin Chem, 113.

[12] De Broe M.E. (1999) Renal injury due to environmental toxins, drugs, and contrast agents, in Berl T, Bonventre JV (eds.): Atlas of Diseases of the Kidney. Philadelphia: Current Medicine, pp. 11.2–11.14.

[13] American Society of Nephrology (2005) Renal research report. J Am Soc Nephrol 16:1886–1903.

[14] Charney D., Solez K., Racusen L.C. (2005) Nephrotoxicity of cyclosporine and other immunosuppressive and immunotherapeutic agents, in Tarloff JB, Lash LH (eds.): Toxicology of the Kidney, 3rd ed., Boca Raton, FL: CRC Press, pp. 687–777.

[15] Hart S.E., Kinter L.B. (2005) Assessing renal effects of toxicants in vivo, in Tarloff JB, Lash LH (eds.): Toxicology of the Kidney, 3rd ed., Boca Raton, FL: CRC Press, pp. 81–147.

[16] Ramos G., Limon-Flores A.Y., Ullrich S.E. (2007) Dermal exposure to jet fuel suppresses delayed-type hypersensitivity: a critical role for aromatic hydrocarbons Toxicol Sci, 100(2):415–422

[17] Schnellmann R.G. (2008) Toxic responses of the kidney In: Casarett and Doull's Toxicology The basic science of poisons Seventh Edition. Editor Curtis D. Klaassen. McGraw-Hill Medical Publishing Division New York, pp. 583-608.

[18] Counts R.S., Nowak G, Wyatt R.D., Schnellmann R.G. (1995) Nephrotoxicants inhibition of renal proximal tubule cell regeneration. Am J Physiol, 269:F274– F281.

[19] Dafour, D.R., 2006. Liver disease. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics Burtis CA, Ashwood ER, Bruns DE (Eds) .4th Edition (2006).